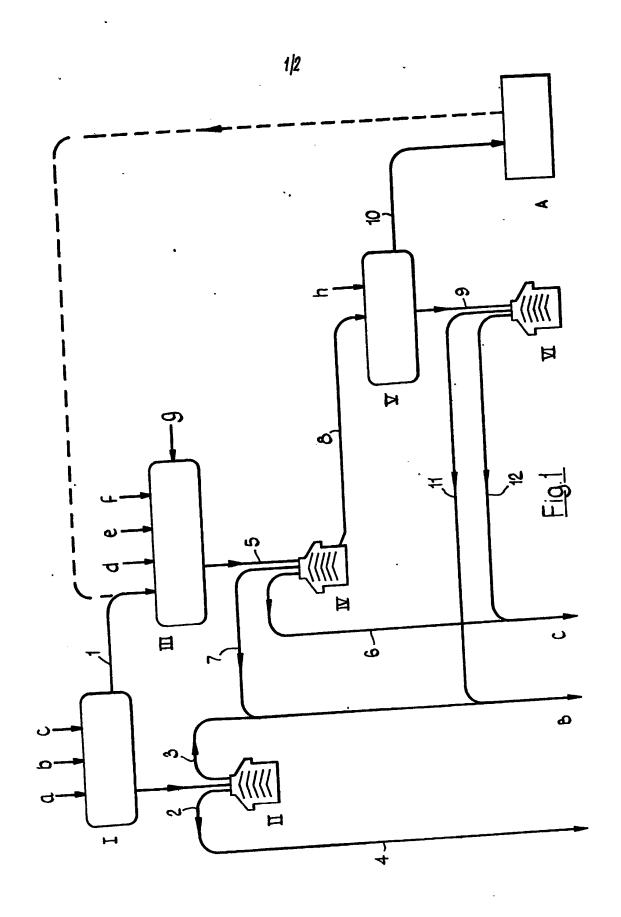
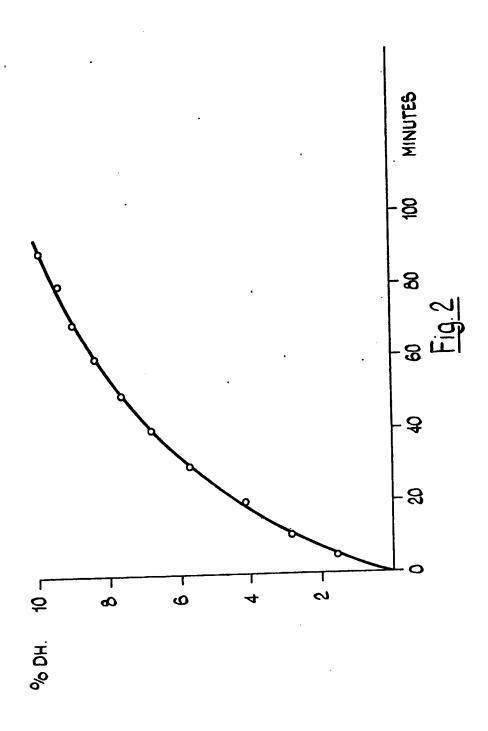
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- (54) Method of producing soy protein hydrolyzate from fat-containing soy material, and such soy protein hydrolyzate
- (57) There is provided a method of producing soy protein hydrolyzate from fat-containing soy material (as defined), which method comprises hydrolyzing a partially defatted solid soy material, obtained by washing a fat-containing soy material in an aqueous medium at a pH in the range of from 3.5 to 5.5 at a relatively constant pH with a proteolytic enzyme in the presence of water and a base to a DH in the range of from 1 to 20 and thereafter deactivating the enzyme, whereafter the aqueous hydrolyzate phase is separated from the oil phase and the solid phase, as well as a product produced by such method.





SPECIFICATION

Method of producing soy protein hydrolyzate from fat-containing soy material, and such soy protein hydrolyzate

The invention comprises a method of producing soy protein hydrolyzate from fat-containing soy mat rial, and soy protein hydrolyzate so prepared.

A method for production of soy protein hydrolyzate from soy beans which are defatted by extraction with organic solvents is described for example in Fifth International Congress of Food Science & Technology, 10 Abstract of paper 3b - 14, "Enzymatic hydrolysis of soy protein. Processing developments and applications in low pH foods". However, due to the presence of fat in the fat-containing soy materials used as a starting material in the present invention and the decisive role which is taken by this fat in all processes, in which fat is present, the invention differs radically from the above indicated production of soy protein hydrolyzate

Soy protein hydrolyzate is a material of growing importance for example for the food industry. Thus, it can from defatted soy beans. be used as one of the main constituent in brines for meat pumping in order to enrich the protein content 15 thereof, as a constituent in soy milk in order to enrich the soy milk with protein without increasing the beany taste normally present in soy milk based on non-hydrolyzed soy material and as a protein enriching agent used as an additive for both acid and neutral soft drinks.

Herein and in the accompanying claims, the term "fat-containing soy material" is used generically to include full-fat soy flour, ground whole soy beans, crushed soy beans, which are partially defatted by mechanical means and similar materials.

Fat-containing soy material, especially full-fat soy flour, is available in huge amounts in areas of the world with industry of a primitive nature.

In any production of a refined protein product with fat-containing soy materials as a starting material, the concomitant fat recovery is important. Usually the recovery of soy oil from soy beans comprises an extraction with organic solvents, generally a hexane extraction. The solvent extraction requires a solvent recovery by fractional distillation, which requires a relatively high investment, and furthermore this process is not ideal from an environmental point of view, especially since ordinarily highly inflammable solvents are 30 used for the extraction. Also, the process is so elaborate that it is not well suited for use at production sites of a primitive nature, for example in developing countries.

Thus, a need exists for a method for treatment of a fat-containing soy material which is well suited for production sites of a primitive nature and which furthermore gives rise to an organoleptically acceptable soy protein hydrolyzate and a considerable recovery of the soy oil and other valuable materials in the full-fat soy

The method for production of soy protein hydrolyzate from fat-containing soy material according to the 35 flour. invention comprises hydrolyzing a partially defatted solid soy material, obtained by washing a fat-containing soy material in an aqueous medium at a pH in the range of from 3.5 to 5.5, at a relatively constant pH with a proteolytic enzyme in the presence of water and a base to a DH in the range of from 1 to 20 and thereafter deactivating the enzyme, whereafter the aqueous hydrolyzate phase is separated from the oil phase and the

A preferred embodiment of the method according to the invention includes the step of washing solid phase. fat-containing soy material, in an aqueous medium having a pH in the range of from 3.5 to 5.5, preferably 4.2

Advantageously, the method of producing soy protein hydrolyzate from fat-containing soy material according to the invention comprises washing the fat-containing soy material (a) in an aqueous medium at a pH in the range of from 4.2 to 4.5 (operation I), the wash water @ from operation I is introduced into a separator, wherein it is separated into an oil phase ③ and a waste water phase ④ (operation II), the washed, partially defatted solid soy material ① from operation I is introduced into a hydrolysis container, to which 50 also water (d), a proteolytic enzyme (e) and base (f) is added, in which hydrolysis container the partially defatted soy material ① from operation I is hydrolyzed at a relatively constant pH to a degree of hydrolysis (DH) in the range of from 1 to 20 (operation III), whereafter the proteolytic activity is inactivated, the slurry (§) from operation III is introduced into a separator, in which the slurry is separated into an oil phase ⑦, an aqueous hydrolyzate phase (a) and a sludge phase (a) (operation IV), the sludge phase (a) from operation IV is collected (product A), the oil phase 3 and 7 from operations II and IV are combined (product B) and the aqueous hydrolyzate phase (6) from operation IV is collected (product C).

The invention also relates to the hydrolyzates produced by the method of the invention.

Surprisingly, it is found that it is possible, according to the invention, by means of a method which is well suited for production sites of a primitive nature to recover in a good yield a valuable soy protein hydrolyzate 60 without bitterness, without soy flavour and without any disadvantageous properties originating from the soy fat which has several application possibilities, around 60% of the oil as a separate oil phase and the precipitate from the hydrolysis, which can be used either as a high grade fodder or as a new starting material

Surprisingly, it has been found that the soy protein hydrolyzate of the invention can be fully acceptable 65 from an organoleptic point of view and also that the oil phase does not turn rancid during the recovery.

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A preferr d embodiment of the m thod according t th inv nti n comprises transporting the sludge phase (8) fr m op ration IV befor collection to a washing device, to which also wat r (h) is added (peration V), whereaft rth precipitat @from operation V is collected as product A, the wash water phase @from operati n V is introduced into a separator, in which it is s parated into an il phase (1) and an aqueous hydro-5 lyzate phas 12 (operati n VI), tho il phases 3, 7 and 11 from pration II, IV and VI ar cmbind (product 5 B) and the aqueous hydrolyzat phases (6) and (2) from operations IV and VI ar combined (product C). In this way, the content of low molecular compounds, for example low molecular peptides, is washed out from the solid phase (8) from operation IV, and the product A will be well suited for repeated hydrolysis. If too many low molecular peptides are present in the material which is subjected to hydrolysis, in the first place these 10 will be decomposed enzymatically to yield a bitter tasting product, and in the second place the proteolytic enzymes will not primarily - as intended - decompose the high molecular soy protein, but rather primarily decompose the low molecular peptides. In a preferred embodiment of the method according to the invention, the separations in one or more or all of operations II, IV and VI are performed by means of centrifuges. In this way, a fast and efficient separation 15 15 is obtained. In a preferred embodiment of the method according to the invention, the proteolytic enzyme used for the hydrolysis is produced by means of Bacillus licheniformis, and that the hydrolysis is performed around the pH optimum of this enzyme. A preferred example of such proteolytic enzyme is the commercial product sold under the Trade Mark "ALCALASE" (subtilisin Carlsberg) by NOVO INDUSTRI A/S. This enzyme is able to 20 split protein along the protein chain with such high hydrolysis rate that the minimal value DH is quickly 20 reached. It is preferred that the hydrolysis is performed at a pH which does not differ more than 2.5 pH units from the optimum pH of the proteolytic enzyme. The optimum pH of the proteolytic enzyme should be determined by means of a substrate related to the hydrolysis mixture. If for example "ALCALASE" is used as the 25 proteolytic enzyme, the enzyme activity curve and thus the optimal pH activity can be determined by means 25 of the modified Anson method described in NOVO Enzyme Information 1B no. 058 e-GB (the original Anson method is described in J. Gen. Physiol., 22, 79-89 (1939)). According to this method, the pH optimum for "ALCALASE" in the hydrolysis mixture is around 9.0 and the pH during hydrolysis should accordingly in this preferred embodiment of the invention have a value in the range of from 6.5 to 11.5. In a preferred embodiment of the method according to the invention, the hydrolysis is carried out to a DH 30 in the range of from 8 to 12. The proteolytic activity is preferably inactivated by means of malic or citric acid. The hydrolysis may be performed in any desired manner, such as that known per se, from the disclosure of United States Patent Specification No. 4,100,024. Also, the soy oil phase can be purified in any desired manner, for instance by the known per se method of 35 removing residual amounts of protein and water. The degree of hydrolysis (DH) is defined by the equation Number of peptide bonds cleaved 100% 40 '40 Total number of peptide bonds Reference is made to J. Adler-Nissen, J. Agric. Food Chem. Vol. 24 No. 6, (1976) page 1090 - 1093, where a more detailed discussion of the definition of DH appears. The number of the peptide bonds cleaved can be measured by means of the ninhydrin method. The 45 ninhydrin method is described in Moore, S., Stein, W.H., "Photometric Ninhydrin Method for use in the Chromatography of Amino Acids", J. Biol. chem., 176, 367-388 (1948). The DH can also be determined if the course of hydrolysis is followed by means of the pH-STAT method, as described in Jacobsen, S.F., Léonis, J., Linderstrøm-Lang, K., Ottesen, M., "the pH-STAT and its use in 50 Biochemistry", in Glick, D, (edit.), "Methods of Biochemical Analysis", Vol. IV. pp. 171-210, Interscience, 50 Publishers Inc., New York (1957). It appears from the above that the DH plays an important role in the invention, in as much as the hydrolysis is controlled by means of the DH: only when DH has reached a critical value, the hydrolysis may be terminated. The DH is, so to speak, the main parameter of the hydrolysis. For a better understanding of the present invention and to show how the same may be put into effect, 55 reference will now be made, by way of example, to the accompanying drawings, in which Figure 1 shows a flow sheet of a preferred embodiment of the method according to the invention, and Figure 2 shows the time - DH relationship pertaining to Example 1. Referring now to Figure 1, the fat-containing soy material (a), which should be pretreated without 60 formation of off-flav ur, is washed (extracted) with water (b) (Operation I). Acid (c) is introduced initially until the pH is in the range of from 4 to 4.5 in the wet soy material, but not later on, because pH turns out to be constant, even if large amounts of water are used. The soy material is washed unti it has a bland taste, and until all soluble materials (at pH 4 to 4.5) are removed. A stepwise operation in which each step includes a separation of the liquid and the solid phase may be used, and if a liquid/solid ratio of 10:1 is used, operation 65 I can be carried out by means of decanter centrifug s or other types of separators. In this case, at least four 65

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steps are found necessary. Other types of extraction equipment may be used, for xampl bask t centrifuges, continuous r batch-operating counter current extractors r press-equipment. By op ration I, a partially defatted and washed soy material ① is removed. Furth rmore, the total amount of wash liquid ② is recovered and, during operation II, this liquid is separated into an oil-phase ③ and an oil fr e phas ④, which may be regarded as waste wat r.

The partially defatted and washed soy material ① is transferred to a hydrolysis tank equipped with a stirrer, thermometer and pH-electrodes connected to a titrator, in which hydrolysis (operation III) takes place. Water ③ is added to the soy material ① until the protein concentration is in the range of from 6 to 10% (N × 6.25). The temperature is adjusted to 50 - 55°C and Alcalase is added (e). If the hydrolyzate is intended for nutritional purposes, a food grade preparation of the proteolytic enzyme is used in such amounts that the total hydrolysis time is around two hours.

The enzymatic hydrolysis (operation III) is carried out at constant pH, preferably at pH 8.0. In order to maintain the chosen pH for the reaction, continuous addition of base (f) is necessary during the reaction. As described in Adler-Nissen Process Biochem. 12(6)18, (1977), the DH can be calculated from the consumption of base (f).

When DH reaches the predetermined value, preferably 10%, the hydrolysis is terminated by addition of acid (g) until the pH is 4.0. The enzyme is inactivated after 30 minutes at pH 4.0 and 50°C. When malic or citric acids are used, the hydrolyzate is non bitter; other acids may be used provided they do not interfere disadvantageously with the product to which the hydrolyzate is supposed to be added.

The finished hydrolyzate (a) is then separated (operation IV) into an oil phase (a), a soy protein hydrolyzate (b) and a sludge phase (b), containing insoluble protein, polysaccharides and residual amounts of oil.

Preferably, a three-phase-centrifuge is used, but a combination of solids ejecting centrifuge followed by a liquid separator is also usable.

The sludge phase (a) is washed (operation V) with water (h) in order to increase the yield of hydrolyzate.
This washin process may be performed as described for operation I. The washed phase (a) (product A) may be further enzyme treated in the same manner as phase (a) or it may be used as animal feed or as raw material for soy source or other fermented products. The wash liquid (a) is separated (operation VI) into an oil phase (b) and an oil free phase (c).

The oil phases ③, ⑦ and ⑪ from operations II, IV and VI are combined to product B from which pure soy bean oil may be isolated.

The oil-free phases (a) and (2) from operations IV and VI are combined as the raw soy protein hydrolyzate C. Product C then may be carbon treated, concentrated and dried, as described in for example United States Patent Specification No. 4,100,024.

The invention is further illustrated by the following Examples.

Fxample 1

600 g of full-fat soy flour (a) (Nutridan TF-100-L from Dansk Soyakagefabrik A/S) having the following composition

Protein (N × 6.25) 43.2% 20.5% Fat 95.0% Dry matter

was stepwise washed at pH 4.2. Each step includes a stirring of the solid phase and water for 30 minutes followed by a centrifugation at 3000 × g for 20 minutes in a laboratory centrifuge (Type Beckmann model J-6B). Results obtained from this washing procedure (operation I) is shown in Table 1, together with the composition of protein (N × 6.25), fat and total dry matter of the partially defatted soy flour and the combined centrifugates from the four steps. Based on these results the mass balance and yields are shown in Table 2. The word "Nutridan" is a Trade Mark, as is the word "Beckmann".

To 666.5 g of the partially defatted soy flour ① (as sludge) which has a pH-value of 4.35 was added 39.6 ml of 4 N NaOH (f) until pH = 8.0, and 1282 g of water (d) was added to dilute the suspension to approximately 8% protein (N × 6.25). The mixture was heated to 50°C in a water bath. 3.20 g of ALCALASE 0.6 L (0.65 Anson units/g) (e) was diluted to 50 ml with water and added to the suspension containing the partially defatted soy flour ①. Thereby an enzyme activity of 13.1 Anson unis per kg protein was obtained. During the hydrolysis pH was kept constant at 8.0 by addition of 4 N NaOH (f) using the pH-stat-method. The degree of hydrolysis was calculated on the bases of the consumption of base (B) by means of the relationship referred to in the reference article of J. Adler-Nissen. The DH-time relationship is shown in Figure 2. At DH = 10% 27.2 ml of 4.0 N NaOH were consumed. Then the hydrolysis was terminated by addition of DL-malic acid (g) until pH = 4.0.44 g of DL-malic acid was used and the hydrolysis was maintained at 50°C for 30 minutes in order to inactivate the enzyme. The hydrolysis mixture was then centrifuged (operation IV) in a laboratory centrifug (Beckmann model J-6B) at 3000 × g for 15 minutes, and 1500 g of centrifugate (h + ⑦), which contains both oil and prot in hydrolyzate, and 554 g of sludge (h) was collected. The sludge phase (h) was washed with 1500 g of water (h) and centrifuged as mentioned above to yield 1500 g of centrifugates (h) + (2) and 500 g of

65 sludge @ (product A) (operation VI). Results obtained after performance of operations III and IV ar shown in

Table 3. After having skimm d the oil phases ⑦ and ⑩ th two centrifugates ⑥ and ⑫ fr m operatins IV and VI, we recombined and adjusted to pH = 5 by use of 4 N NaOH (amount not determined) and activated carbon (BGN from Lurgi Apparate-Tichnik) was added in an amount of 0.2% of the total volume of hydrolyzate. After stirring for 30 minutes at 50°C the activated carbon was removed by filtration through glass fibre filter (Watman glass fibre GF/F) which has previously been washed with 5 litre of deidenized water, in order to remove off-flavours from the filter. The filtrate was adjusted to pH = 6.5 and diluted to 4% protein (N × 6.25) before evaluation by means of a trained taste panel consisting of 14 persons. The hydrolyzate was compared with a sample produced from defatted flakes, as described in e.g. Fifth International Congress of Food Science & Technology, Abstracts of paper, 3b-14, "Enzymatic hydrolysis of soy protein. Processing development and applications at a low pH foods". A triangle-taste-evaluation was performed resulting in seven right answers and seven wrong answers, indicating that a taste difference could not be deomonstrated. The word "Watman" is a Trade Mark.

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TABLE 1

Centrifugate and solid phase related to operation I.

		1. step	2. step	3. step	4. step	Combined or final
Full-fat soy flour 6 N HC 1 Water	(a) (a)	600 34.5 6000	0009	00009	0000	1 1 1
<i>Centrifugate:</i> Mass Protein conc., N × 6.25 Dry matter Fat	(% (% (%) (%) (%) (%) (%) (%)	5160 0.25 2.22 · not determ.	5300 0.07 0.49 not determ.	5000 0.07 0.23 not determ.	5000 0.04 0.15 not determ.	20460 0.10 0.78 0.20
Solid phase: Mass Protein conc. N × 6.25 Dry matter Fat	(6) (8) (8)	1050.6 not determ. - " -	991.4 not determ. - " -	1032.0 not determ. - " -	1009.7 23.9 40.7 8.2	1009.7 23.9 40.7 8.2

TABLE 2

•		Mass balance	e and yield	ls related to operation	on I		
5	·	Fuil-fat soy flour		C mbined centri- fugate	det flo	rtially fatted soy ur (as dge)	5
10	Total mass (g)	600		20460	100	09.7	10
15	Mass of dry matter (g) Yield (%)	570.1 100		159.6 28.0		10.9 72.1	15
	Mass of pro- tein (g) Yield (%)	259.1 100		20.5 7.9		11.3 33.1	
20	Mass of fat (g) Yield (%)	123 100		40.9 33.3		32.8 57.3	20
25			TAB	LE 3			25
		Results obtained at	ter perform	nance of operations	III and IV.		
30	Process step and fraction	Mass of fraction, g	% Pro- tein	Yield of protein	% Fat	Yield of fat %	30
35				Based on partially defatted flour/based on full fat flour		Based on partially defatted four/based on full fat flour	35
40	Operation III						40
45	Partially defatted soy flour	666.5	23.9	100/93.1	8.2	100/67.3	45
	After hydrolysis	2117.8	7.5	100/93.1	2.6	100/67.3	
50	Operation IV. Centrifu- gate ① + ⑥	1500	4.3	40.6.37.8	1.2	32.7/ 22.2	50
55	Sludge ®	554	not ar	nalysed	not a	nalysed	55
	Operation V Centrifu- ① +	1500	not ar	nalysed	not a	nalysed	
60	@						60
65	Product A	500	14.3	44.9/41.8	7.3	66.8/45.0	65

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Example 2

20 kg of full-fat soy flour (a) (Nutridan TF-100 L from Dansk Soyakagefabrik A/S) having the composition indicated in Example 1 was stepwise washed at pH = 4.2 using 4 × 180 1 fwater (b) of 15-20°C (operation I), acid (c) being introduced into the first step inly. Each step includes a stirring of the solid phase and water 5 foll wed by a centrifugation in a d cant r centrifuge (Alfa-Laval N × 310-B). The sludg content in the centrifugate (determined after centrifugation of 10 ml in a graduated tube) was 2-4%. Therefore the centrifugate was re-centrifuged in a solids ejecting centrifuge Westfalia (SB 7-35-076). The results are shown in Table 4. The centrifugate indicated in Table 1 was the centrifugate from the Westfalia centrifuge and the sludge was the combined (total) sludge from the decanter and the solids ejectig centrifuge (operation I). The 10 total combined 630 liters of centrifugates ② were separated into 2.8 kg of an oil phase ③ and 627 kg of an oil-free phase (4) using a Westfalia centrifuge of type LG 205-2. The results obtained are shown in Table 5. The word "Westfalia" is a Trade Mark. Based on these results the mass balance and yield related to operations I and II are shown in Table -6. To 41 kg of the partially defatted soy flour (1) was added 46 kg of water (d) to dilute the sludge to about 15 6.75% protein, 685 ml of 4.8 N NaOH was added to adjust the pH to 8.0. The mixture was stirred and heated to 55°C in a tank with heating mantle. 118 g of Alcalase 0.6 L (0.65 Anson units/g) (e) was diluted to 5 liters with cold water and added to the suspension. During the hydrolysis, pH was kept constant at 8.0 by addition of 4.8

cold water and added to the suspension. During the hydrolysis, pH was kept constant at 8.0 by addition of 4.8 N NaOH (f) using the pH-stat-technique. A DH of 10% was reached after 133 minutes when 843 ml of 4.8 N NaOH has been consumed. Immediately thereafter, 1887 g DL-malic acid (g) was added to give a pH of 4.0.

The suspension was kept stirred at 30 minutes in order to inactivate the enzyme (operation III).

The hydrolysis mixture was then centrifuged in the solids-ejecting centrifuge (Westfalia SB 7-35-076) and

The hydrolysis mixture was then centrifuged in the solids-ejecting centrifuge (Westfalia SB 7-35-076) and 37 ℓ of centrifugate (6) + (7) was recovered together with 50 ℓ of diluted sludge (8). The centrifugate was then separated into 84 g of oil (7) and 34 litres of oil-free phase (6) (operation IV).

The sludge (a) was washed with 70 litres of water (h) (operation V) and separated into 73 litres of sludge (a) and 45 litres of wash liquid (a) which was separated into 43 litres of oil-free phase (a) and 66 g of oil (b) (operation VI). Results obtained during the recovery of soy protein hydrolyzate are shown in Table 7.

The oil-free hydrolyzates (6) and (2) were combined (product C), filtered, carbon treated, concentrated by reverse osmosis and freeze dried.

The oil-phases ② and ① were combined with the oil phase ③ from operation II, giving rise to product B. The composition and yields of the combined products A, B and C are shown in Table 8.

It appears from the following Tables that the accuracy of the mass balances is not complete. This is due to the inaccuracy which accompanies weighing and measuring of small amounts in relatively large equipment.

TABLE 4

Centrifugate and solld phase related to operation l

Combined or final	720	630 0.25 0.96 0.50	51.5 14.19 21.77 1.80
4 step	180	156 0.13 0.21 0.20	61.5 14.19 21.77 1.80
3. step	. 1 1 1	160 0.13 0.50 Not determ.	51.8 15.06 22.95 not determ.
2. step	. 1 80	160 0.13 0.43 0.18	57.4 16.0 27.7 · not determ.
1. step	20.0 1.3 180.0	155 0.38 3.30 1.23	59.3 16.44 27.51 4.12
	(kg)	(kg) (%) (%)	(kg) (%) (%)
	Full-fat soy flour 6 N HCI Water	Centrifugate: Mass Protein (% N × 6.25) Dry matter Fat	<i>Solid phase:</i> Mass Protein (% N × 6.25) Dry matter Fat

TABLE 5

Results obtained after performance of operation II

Oil-free phase @	627	0.19	0.77 not determ.
Oil phase ®	2.8	1.44	62.4 59.6
Combined centrifugate ②	630	0.25	0.96 0.50
	Mass (kg) Protein	(% N × 6.25) Dry matter	(%) Fat (%)

TABLE 6

Mass balance and yields related to operations I and II.

		Operation	_	Operation II		
		Full-fat soy flour (a)	wash liquid ©	Partially de- fatted soy flour⊕	Oll-phase	Oll free phase@
Totai mass	(kg)	20.0	630	51.5.	8.	627
Mass of DM Yield	(kg) (%)	100	6.05 31.8	11.2 59.0	1.75 9.2	4.83 25.4
Mass of protein Yield	(kg)	8.64	1.58 18.5	7,31 84.6	0.04	1.19
Mass of fat Yield	(kg) (%)	4.10 100	3.15 76.8	0.92 22.4	1.67 40.7	not determ.

() means that the figures is unrealistic.

TABLE 7

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s III, IV,
operation
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performance
after
obtained
Results

	Mass of fraction kg	•	Yield of protein % based on base partially fulldefatted flour	tein % based on full-fat flour	% fat	Yield of fat based on ba partially ful defatted flo flour	f fat based on full-fat flour
95.5 5.5		. 6.56	9 - 1	88 I 6.	1.80 (1.67)	100	- 22.4
0.084 34		2.63 4.38	, 25.6	0.03	60.4	 69 I	ر تن
20		7.63	65.6		not ana- lyzed	ı	1
73		2.75	34.6	. 29.2	not ana- iyzed	1	ı
45	-	1.88	14.5	12.3	not ana- lyzed	ı	1
0.066 43		2.63	0.03 13.4	0.02	61.5	ا ق ا	1.2

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TABLE 8

Composition and yields of product A. B and C based on full-fat soy flour

	Composi	uon and ylelus ol produ	ict A, B and C pased	on Tull-Tat Soy Tigur	
5	Compon nt	A .	В	· c	5
	Protein %	2.75	1.5	2.99	
	Yield %	29.2	0.5	33.0	
10					10
	Dry matter	10.3	65	4.5	
	%			-	
	Yield %	(84.2)	10	22.9	
15	Oil %	not determ.	60	_	15
	Yield %	not determ.	43.4	-	
	() means that the f	igure is unrealistic.			
20 CI	AIMS				20

A method of producing soy protein hydrolyzate from fat-containing soy material (as defined), which
method comprises hydrolyzing a partially defatted solid soy material, obtained by washing a fat-containing
soy material in an aqueous medium at a pH in the range of from 3.5 to 5.5 at a relatively constant pH with a
proteolytic enzyme in the presence of water and a base to a DH in the range of from 1 to 20 and thereafter
deactivating the enzyme, whereafter the aqueous hydrolyzate phase is separated from the oil phase and the

deactivating the enzyme, whereafter the aqueous hydrolyzate phase is separated from t solid phase.

A method according to Claim 1, which includes the step of washing fat-containing soy material in an aqueous medium having a pH in the range of from 3.5 to 5.5.

A method according to Claim 2, wherein the step of washing the fat-containing soy material is washed
in an aqueous medium having a pH in the range of from 4.2 to 4.5.

4. A method for production of soy protein hydrolyzate from fat-containing soy material, wherein the fat-containing soy material (a) is washed in an aqueous medium at pH 3.5 to 5.5 (operation I), preferably from 4.2 to 4.5, whereby the wash water ② from operation I is introduced into a separator, wherein it is separated into an oil phase ③ and a waste water phase ④ (operation II), and whereby the washed, partially defatted, solid soy material ① from operation I is introduced into a hydrolysis container, to which also water (d), a proteolytic enzyme (e) and base (f) is added, in which hydrolysis container the partially defatted soy material ① from operation I is hydrolyzed at a relatively constant pH to a DH of between 1 and 20 (operation III), whereafter the proteolytic activity is inactivated, whereby the slurry ⑤ from operation III is introduced into a separator, in which the slurry is separated into an oil phase ⑦, an aqueous hydrolyzate phase ⑥ and a sludge phase ⑧ (operation IV), whereby the sludge phase ⑧ from operation IV is collected (product A), whereby the oil phases ③ and ⑦ from operations II and IV are combined (product B) and whereby the aqueous hydrolyzate phase ⑥ from operation IV is collected (product C).

5. A method according to Claim 4, wherein the sludge phase (a) from operation IV before collection is transported to a washing device, to which also water (h) is added (operation V), whereafter the precipitate (b) 45 from operation V is collected as product A, the wash water phase (a) from operation V is introduced into a separator, in which it is separated into an oil phase (b) and an aqueous hydrolyzate phase (a) (operation VI), the oil phases (a), (b) and (b) from operations II, IV and VI are combined (product B) and the aqeuous hydrolyzate phases (a) and (b) from operations IV and VI are combined (product C).

A method according to Claim 4 or 5, wherein the separations in operations II and IV or II, IV and VI are
performed by means of a centrifuge.

7. A method according to Claim 1 or 6, wherein the proteolytic enzyme (e) used for the hydrolysis is produced by means of *B. licheniformis* and the hydrolysis (operation III) is performed around the pH optimum of this enzyme.

8. A method according to Claim 1 to 7, wherein the hydrolysis (operation III) is performed at a pH which does not differ more than 2.5 pH units from the optimum pH of the proteolytic enzymes.

9. A method according to Claim 1 to 8, wherein the hydrolysis (operation III) is carried out to a DH in the range of from 8 to 12.

10. A method according to Claim 1 to 9, wherein the proteolytic activity is inactivated by means of malic 60 or citric acid.

11. A method of producing soy protein hydrolyzate substantially as hereinbefore described with refer nce to Figure 1 of the accompanying drawings.

12. A method of producing soy protein hydrolyzate substantially as hereinbefore described with reference to Figure 2 of the accompanying drawings.

; 13. A method of producing soy protein hydrolyzat substantially as described in any one of the foregoing 6

Examples.

- 14. A soy prot in hydrolyzate whenev r produced by the method of any ne f the preceding claims.
- 15. Any novel featur or combination of features described herein.

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